MechanicalDiversity of Porous Poly (Ethylene Glycol) Diacrylate

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Abstract

This study investigates the effects of polymerchain length on the mechanical properties of gas foamed poly (ethylene glycol) diacrylate (PEGDA). Gas foamed structures were composed of PEGDA MW 700, MW 3400, or MW 10k. Combinations of short (lower MW) and long PEGDA (higher MW) chains created polymer structures with increased strength and stiffness compared to structures composed of a given longer PEGDA chain. Gas foamed PEGDA hydrogels are structurally and mechanically suitable for evaluating cells in 3D culture. SEM images show an interconnected pore structure. Gas foamed PEGDA is also cytocompatible. High cell viability was observed on collagen type I infused polymer structures. Changes in PEGDA chain sizesneither degraded cell viability nor impeded gas foaming. The mechanical tunability of gas foamed porous PEGDA makes it an effective tool for evaluating cellular response to static mechanical properties in 3D.

Keywords

PEGDA; Mechanical Stability; Collagen Network; Tissue Engineering

Introduction

Tissue engineering constructs made with synthetic polymers allow users to control the mechanical and structural properties of the device. Poly (ethylene glycol) diacrylate (PEGDA) is a synthetic polymer that has been used to investigate the engineering of tissues including bone (Yang, et al. 2005), cartilage (Gabler, et al. 2009) (Hwang 2007), and cornea (Kadakia, Keskar, et al. 2008) (Myung, P.E., et al. 2008).Previous publications have demonstrated its amenability to chemical, structural, and mechanical modification. (Safranski, et al. 2011) (Lee, Lee and Koh 2007) (Keskar, Marion, et al. 2009) (Lin, et al. 2011). In addition, PEGDA is non-toxic (Mazzoccoli, et al. 2010) and generates minimal immunogenic response (Lynn, Kyriakides and Bryant 2010).The mechanical tunability of gas foamed PEGDA may give it a range of tissue engineering applications.

TypicalPEG hydrogel studies use a singular PEG chain size to adjust the structure's mechanical properties. (Lin, et al. 2011) (Myung, Koh, et al. 2007) (Sannino, et al. 2006) This study investigated thecombination of multiple PEGDA chain sizes to develop gas foamed PEGDA hydrogels with different mechanical properties.Variations in PEGDA molecular weight or solution concentration change the kinetics of polymerization and alter mechanical properties of the gel. (Lee, Lee and Koh 2007) (Mazzoccoli, et al. 2010) (Beamish, et al. 2010) (Pfister, et al. 2007)Increasing the ratio of PEGDA to water, at the time of polymerization, or decreasing poly(ethylene glycol) (PEG) chain length leads to increased mechanical modulus and decreased mass transport through the gel. (Beamish, et al. 2010)Since PEGDA alone is known to resist cell adhesion in vitro (Kadakia, Keskar, et al. 2008) (Hern and Hubbel 1998), rat tail collagen type I was added to porous PEGDA to permit cell attachment. Throughout this report, the combination of porous PEGDA and rat tail collagen type I is called a hybrid scaffold. This study demonstrates that by simply combining PEGDA chains of given lengths, one can adjust the mechanical properties of porous PEGDAstructures that are suitable for tissue engineering.

Methods

Engineering theHybrid Scaffold

Porous PEGDA scaffolds were designed based on theprotocol published by Keskaret. al. (Keskar, Marion, et al. 2009). PEGDA MW 10k (Sigma-Aldrich), PEGDA MW 3400 (Glycosan, Salt Lake City, UT), or PEGDA MW 700 (Sigma-Aldrich) were used to create gas foamed PEGDA. To prepare the polymer structures for in vitro evaluation, the structures were immersed in a

rat tail collagen type I solution (2 mg/ml) (BD Bioscience). N (3-Dimethylaminopropyl)–N'–ethyl-carbodiimide (EDC) (5 mM) and N-hydroxysuccinimide (NHS) (5 mM) were used to crosslink the collagen. Dehydrated porous PEGDA structures absorbed the collagen solution like sponges. After the collagen type I solution absorbed, it was gelled at 37°C. The collagen gel is much weaker than the porous PEGDA structure; (Kadakia, Keskar, et al. 2008), fails to contribute to the overall mechanical properties of PEGDA, and unlikely alters the pore size.he combination of porous PEGDA and collagen type I isreferred to as the hybrid scaffold throughout this report. Surface pore areas were measured using SEM images. Image *J* applied a threshold to surface pores and measured each pore with the Analyze Particles function.

Tensile Testing of Porous PEGDA

Hydratedporous PEGDAsamples were tested in tension using a custom designed 100LM Test Resources mechanical testing machine (Test Resources Inc., Shakopee MN). Three samples of each formulation were tested using a calibrated WF75GS Load Cell (Test Resources Inc.), which is fatigue-rated for 75 g of force (0.735 N) in tension and compression. The scan rate for all tension measures was 50.86 Hz. Samples were cut into rectangular shapes for tensile testing.Average sample dimensions were approximately 4 x 8 mm and 3 mm depth. The tensile strain rate was 0.2 mm/s. Firstly, a strain of 10% was applied for at least 30 cycles to pre-condition the samples. A strain of 200% was applied to measure stress vs. strain to the rupture point.Values taken from the tensile measures included elastic modulus (E), ultimate tensile strength (UTS), and strain at rupture. *E* is measured in the elastic or linear region of the stress vs. strain plot; *E* = stress/strain. UTS are the maximum stress response of a material during tensile testing. The strain at rupture is simply the elongation of the sample at the point of rupture; in this study the elongationwas reported as a percentage of the original length of the sample.

Structural Evaluation

To observe the pore structure of scaffolds, SEM images of polymers were generated using a S-3000N Variable Pressure SEM (Hitachi). Surface pore areas were measured using SEM images. Image *J* applied a threshold to surface pores and measured each pore with the Analyze Particles function. Monoclonal Anti-Collagen Type I antibody at 1: 2000 dilution (Sigma-Aldrich, C2456) was used to stain collagen fibers in the hybrid scaffolds.

Cell Culture and Cell Viability

Two cell types were used to evaluate in vitro cytotoxicity and cell proliferation: primary human corneal fibroblasts (HCFs) passage 11 and human fibroblast cell line (HT1080) passage 7. Cellswere cultured in GIBCO MEM alpha media (Invitrogen) with 10% fetal bovine serum (FBS) (Atlanta Biologicals, Lawrenceville, GA) and 1% antibiotic-antimycotic solution (Sigma-Aldrich). A live-dead cell viability assay (Invitrogen) was used to stain live and dead cells in hybrid scaffolds.

DNA Assay

Acellularhybrid scaffolds, approximately 0.5 –1 mm in thickness and 7 mm in diameter, were placed on a 50-60% confluent layer HCFs and incubated for periods of 7, 9, and 13 days. Hybrid scaffolds were rinsed in PBS prior to DNA isolation to ensure that only cellsattached to the scaffold were evaluated. TRIzol Reagent (Invitrogen) was used per the product protocol to isolate DNA. DNA pellets were diluted with 250 μL of TE Buffer, and a fluorescent DNA quantification kit (BioRad 170-2480, Hercules, CA) was used to measure the DNA concentration. For each sample, 80 μL of the prepared fluorescent DNA solution was added to a well. In all, 6 wells were measured for each DNA pellet;and four DNA pellets were tested (n = 4).

Statistical Analysis

MATLAB R2012a was employed for all statistical analysis using an alpha value of 0.05. Note that the standard error of a given data set represents the error measure. This report references the MATLAB R2012a Product Documentation to discuss the application of MATLAB statistical tests.

Results

Mechanical and Structural Assessment

Various quantities of PEGDA MW 700, 3400, or 10k were combined to create porous PEGDA scaffolds with different mechanical properties. Monomer combinations resulted in stiffness values ranging from a weak jelly-like softness to a glass-like hardness (Fig. 1). The grams per milliliter (g/ml)ofPEGDA chainsused and the equivalent molar concentrations

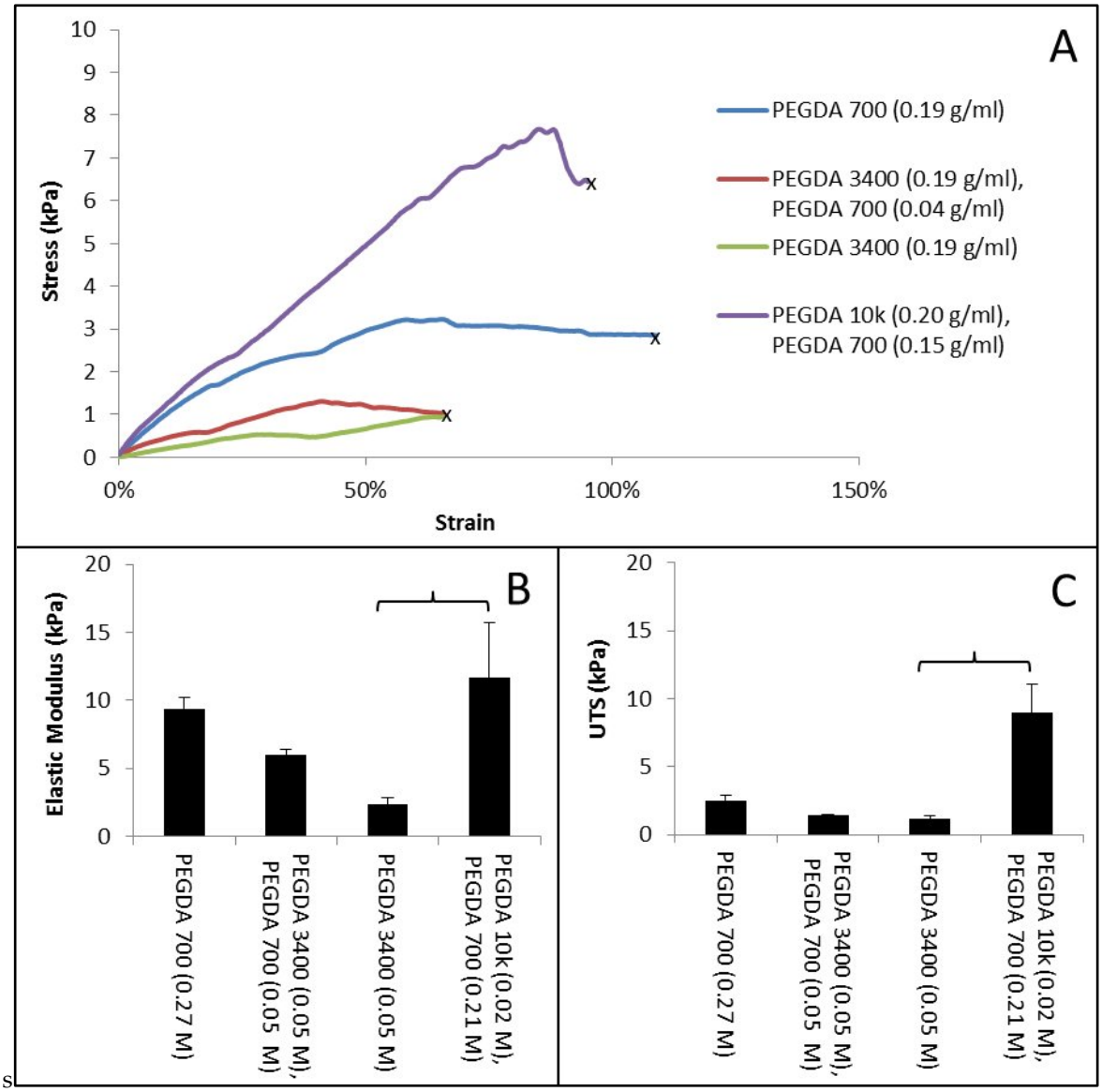


FIGURE 1 THE COMBINATION OF PEGDA CHAIN LENGTHS OR MWS DETERMINED THE MECHANICAL PROPERTIES OF EACH POROUS SUBSTRATE. IN COMPARISON TO INCREASING PEGDA 700 CONCENTRATION ALONE, COMBINING PEGDA 10K AND PEGDA 700 CHAINS APPEARED TO BE A MORE EFFECTIVE STRATEGY FOR INCREASING HYDROGEL STRENGTH AND STIFFNESS. THE VISCOSITY OF HIGH CONCENTRATION PEGDA 3400 SOLUTIONS, AND OTHER CONCENTRATED SOLUTIONS CONSISTING OF LONGER PEGDA CHAINS, CAN INHIBIT CHEMICALLY INITIATED GAS FOAMING; THE COMBINATION OF PEGDA 700 AND PEGDA 3400 CHAINS SHOWED THAT MIXTURES OF PEGDA CHAIN SIZES ARE USEFUL FOR THE MECHANICAL MODULATION OF POROUS PEGDA HYDROGELS. PEGDA SOLUTIONS THAT WERE USED TO CREATE THESE HYDROGELS WERE FLUID ENOUGH TO PERMIT CHEMICALLY INITIATED GAS FOAMING. THE COMPOSITION OF EACH SAMPLE IS LISTED IN G/ML OR IN ITS EQUIVALENT MOLAR CONCENTRATION. THE SUBSTRATES' STRESS RESPONSE TO THE POINT OF FAILURE CHARACTERIZED THEIR OVERALL STRENGTH AND DUCTILITY (A);AND 'X'MARKS THE POINT OF RUPTURE FOR EACH SAMPLE. THE COMBINATION OF PEGDA MW 10K AND 700 YIELDED A SIGNIFICANTLY STRONGER (B) AND STIFFER (C) POROUS SUBSTRATE COMPARED TO THE PEGDA MW 3400 SAMPLE. COMPARED TO PEGDA 3400, THE INCREASED STIFFNESS AND STRENGTH OBTAINED BY COMBINING PEGDA MW 700 AND PEGDA 3400 WAS NOT STATISTICALLY SIGNIFICANT

(M) are listed for each polymer samplein Figure 1;and these samples were tested at a strain rate of 0.2 mm/s.Per ANOVA testing, the UTS and E of PEGDA 10k/700 are significantly greater than the UTS and *E* for PEGDA 3400. Trends in mechanical data show that the addition of PEGDA 700 to structures increased strength (UTS) and stiffness (E). A porous sample composed of PEGDA 10k (0.20 g/ml or 0.02 M) and PEGDA 3400 (0.07 g/ml or 0.02 M) was also tested in tension. However, the force response obtained from the combination was too small and noisy to extract reliable mechanical data. Porous PEGDA 10k (0.20 g/ml or 0.02 M) was too weak and unstable to test in tension, which had the consistency of jelly or a weak

gum-like substance. The specific combination of PEGDA chain lengths determined the mechanical properties of each porous PEGDA substrate.

Combiningsmall quantities of different chain sizes can result in porous PEGDA hydrogels withdifferent mechanical properties (Fig. 1). The addition of PEGDA 10k (0.02 M) to PEGDA 700 (0.21 M) resulted in a structure that was stronger than PEGDA 700 made with a higher concentration of monomer (0.27 M). The combination of PEGDA 3400 (0.05 M) and PEGDA 700 (0.05 M) created a hydrogel that was stiffer than PEGDA 3400 (0.05 M). The mechanical tuning of hydrogels via PEGDA chain size combinations can be advantageous when viscous single size chain solutions inhibit chemical gas foaming. Instead of increasing monomer concentration, thereby increasing solution viscosity, different chain sizes can be combined to alter the mechanical properties of porous PEGDA hydrogels. The PEGDA chain solutions used to make hydrogels (see Fig. 1) were fluid enough to add a high density of pores via chemically initiated gas foaming. Representative SEM images of gas foamed PEGDA 3400 (Fig. 2A) and PEGDA 10k/700 (Fig. 2B) show interconnected pores. Surface pores of PEGDA 3400 and PEGDA 10k/700 are 65 ± 4 and 46 ± 3 μm in diameter, respectively. The ranges of surface pore diameters are 6 – 317 and 6 – 214 μm for PEGDA 3400 and PEGDA 10k/700, respectively. The pore structure is important because it provides space for cells to grow into the body of the hydrogel structure.

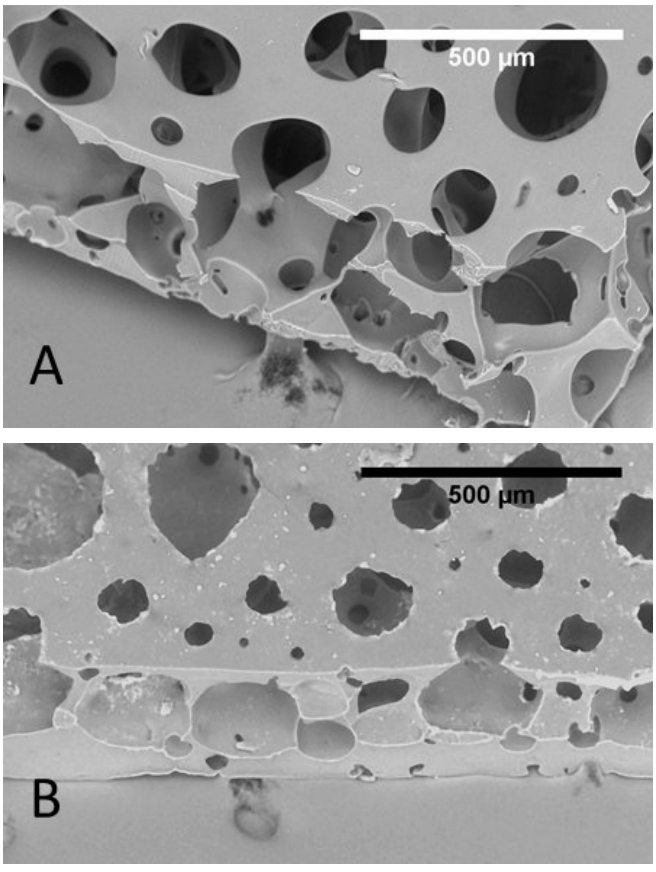


FIGURE 2. SEM IMAGES OF PEGDA 3400 (A) AND PEGDA 10K/700 (B) SHOW THAT GAS FOAMING OF PEGDA CREATES AN INTERCONNECTED PORE STRUCTURE IN THE POLYMER

In Vitro Evaluation

The hybrid scaffolds permit cell migration and a high level of cell viability. The collagen type I network in the hybrid scaffold is shown in Figure 3. The collagen type I permits cell adhesion on the structure. At day 4 of cell migration, the majority of HT 1080s, passage 7, were viable in hybrid scaffolds made with PEGDA 3400 and PEGDA 10k/700 (Fig. 4). Calcein-AM strained viable or live cells green. Ethidiumhomodimer strained dead cells red; it also stains PEGDA at a lesser intensity compared to dead cells. HCF, passage 11,migration into hybrid PEGDA10k/700scaffoldsincreased significantly from day 7 to day 13 (Fig. 5) per Kruskal-Wallis testing.

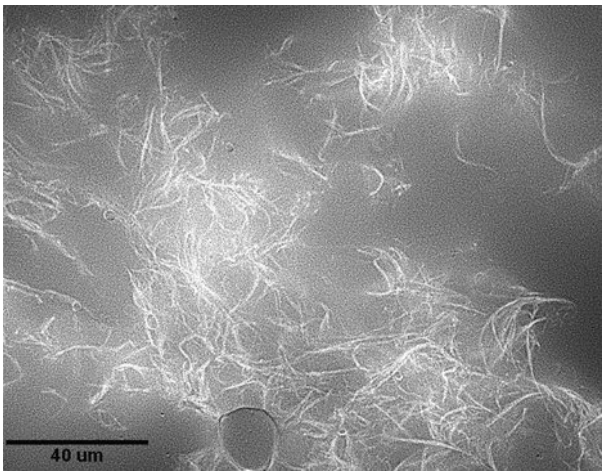
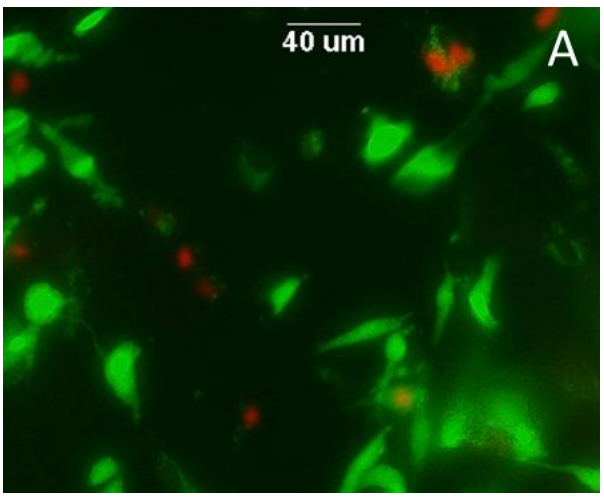


FIGURE 3. COLLAGEN TYPE I WAS ADDED TO POROUS PEGDA TO AID CELL SPREADING. THE DISPLAYED FIBERS WERE STRAINED WITH ANTI-COLLAGEN TYPE I ANTIBODY



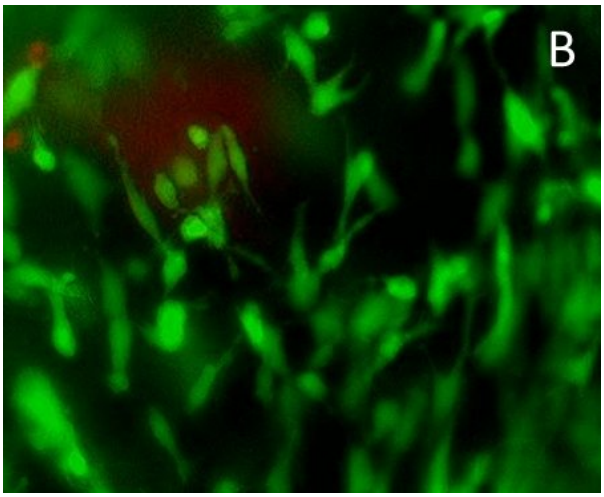


FIGURE 4 A HUMAN FIBROBLAST CELL LINE (HT 1080), PASSAGE 7, WAS USED TO EVALUATE THE CYTOTOXICITY OF POROUS PEGDA INFUSED WITH COLLAGEN TYPE I. STRUCTURES MADE WITH PEGDA 3400 (A) AND PEGDA 10K/700 (B) WERE STAINED TO IDENTIFY LIVE AND DEAD CELLS; BOTH IMAGES WERE TAKEN AT 20X. CELLS MIGRATING INTO THE STRUCTURES OVER 4 DAYS SHOWED A HIGH LEVEL OF VIABILITY. CALCEIN-AM STRAINED LIVE CELLS GREEN. ETHIDIUMHOMODIMER STRAINED DEAD CELLS RED; IT ALSO STAINS PEGDA AT A LESSER INTENSITY COMPARED TO DEAD CELLS

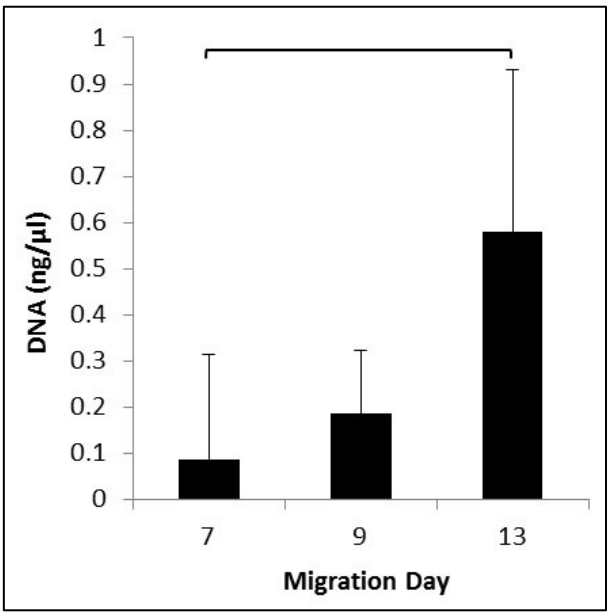


FIGURE 5 INCREASED DNA CONCENTRATIONS SHOW THAT PRIMARY HUMAN CORNEAL FIBROBLASTS (HCF) MIGRATED INTO THE POROUS PEGDA 10K/700 COATED WITH COLLAGEN TYPE I (N = 4). PER KRUSKAL WALLIS TESTING, THERE WAS A STATISTICALLY SIGNIFICANT INCREASE IN CELLS FROM DAY 7 TO 13. THE HORIZONTAL BAR NOTES THE STATISTICALLY SIGNIFICANT RELATIONSHIP BETWEEN THE TWO TIME POINTS

Discussion

This study demonstrates that the mechanical properties of gas foamed PEGDA can be adjusted while preserving cytocompatibility and without sacrificing pore interconnectivity.Previous studies showed that PEGDA and the collagen type I infused into gas foamed PEGDA are cytocompatible. (Kadakia, Hybrid Superporous Scaffolds: An Application for Cornea Tissue Eingineering 2008) (Keskar, Marion, et al. 2009) (Keskar, Gandhi, et al. 2009)Collagen type I is known to support the growth and development of fibroblasts in vitro and in vivo. (Nishida, et al. 1988) (Duncan, et al. 2010) (Phu and Orwin 2009)Keskaret. al. demonstrated that gas foamed PEGDA supports human mesenchymal stem cell (hMSC) growth for approximately 4 weeks. (Keskar, Marion, et al. 2009)Zhu et. al. reported smooth muscle cell growth on PEGDA with cell adhesion components. (Zhu, et al. 2006)Lin et. al. showed that chondrocytes encapsulated in PEGDA can proliferate for up to 4 weeks. (Lin, et al. 2011). A variety of cell types thrive on PEGDA scaffolds. This suggests that gas foamed PEGDA could potentiallyserve as a 3D structure for a variety of cell types.

Gas foamed PEGDA structures are designed to mimic the interconnectivity of natural ECM. Interconnected pores in gas foamed PEGDA allow cells to migrate throughout the body of a porous tissue engineering structure. (Keskar, Gandhi, et al. 2009) (LaNasa 2011)SEM images (Fig. 2) show that the polymer chain lengths of gas foamed PEGDA can be adjusted while maintaining an interconnected pore structure. A previous study supported this finding that pore morphology remained relatively constant when different chain lengths were used to create gas foamed PEGDA. (Sannino, et al. 2006)Given the interconnected pore structure that permits cell ingrowth, the adjustable mechanical properties of gas foamed PEGDA could potentially aid the investigation of cell response to ECM mechanics in 3D. Keskaret.al. showed that the pore structure in gas foamed PEGDA facilitated large scale cell ingrowth in mice; however, tissue ingrowth into non-porous hydrogels was not observed. (Keskar, Gandhi, et al. 2009) The preservation of pore interconnectivity following mechanical adjustment is imperative for cell growth throughout the body of the 3D structure.

Adjusting PEGDA chain lengths is an efficient method for modulating the mechanical properties of a gas foamed PEGDA. Other laboratories have reported the effects of polymer chains sizes on the mechanical properties of polymers. (Myung, Koh, et al. 2007) (Lin, et al. 2011) Compression testing of blends of PEGDA MW 3400 and PEGDA MW 400 showed that different ratios of PEGDA chain sizes result in different stiffness

or elastic modulus values. (Mazzoccoli, et al. 2010)Myunget. al. used poly (ethylene glycol) (PEG) chains ranging from 3400 to 14000 MW to adjust the mechanical properties of interpenetrating polymer networks composed of PEG and poly(acrylic acid) (PAA). (Myung, Koh, et al. 2007)The mechanical properties of a tissue engineering structure can guide cell behavior and astructure's long term survival depends on the mechanics of in vivo implantation site. (Guldberg, et al. 2008) (Engler, et al. 2006) (Ghosh, et al. 2007) (Hacker and Mikos 2011) (Dunn 2006)

The strength and stiffness of a PEGDA tissue engineering structure can be enhanced by increasing the quantity of a single PEGDA chain type. (Beamish, et al. 2010) (Mazzoccoli, et al. 2010) However, concentrated aqueous PEGDA solutions can be viscous, especially when larger chain lengths are used, and highly viscous solutions can reduce the efficacy of the chemically initiated gas foaming method described in this paper. Monomer solutions used to make hydrogels (see Fig. 1) were fluid enough to permit gas foaming. Mechanical tuning using combinations of PEGDA chain sizes is a novel alternative to viscous single size chain solutions that inhibit the chemical gas foaming process. The interconnected pore network introduced by gas foaming is essential for potential 3D cell development in PEGDA.

Conclusions

The mechanical properties of gas foamed porous PEGDA depend in part on polymer chain length. After testing multiple combinations of PEGDA chain lengths or molecular weights, we determined that the presented polymer fabrication method can be used to producecytocompatible structures with interconnected pores and a range of mechanical properties. This mechanically tunable porous structure provides a 3D structure for observing the cell's response to substrate mechanics.

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